EXPERIMENTS ON THE PHYSICAL STUCTURE OF THE PROTOPLASM OF PARAMŒCIUM AND ITS RELATION TO THE REACTIONS OF THE ORGANISM TO THERMAL, CHEMICAL AND ELECTRICAL STIMULI.

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CONTENTS.

INTRODUCTION.

It has been known for several years that a marked similarity in physical structure exists between protoplasm and that class of chemical solutions known as colloidal solutions. This similarity was pointed out by Hardy in 1899 as a result of his investigations upon the physical structure of certain organic colloids, as egg albumen, gelatin, etc., which resemble protoplasm very closely in their gross appearance, and of observations upon protoplasm itself under various conditions.

The conclusions of Hardy and others[1] in regard to the physical structure of the organic colloids are of such importance in the further development of this paper, and are so largely unappreciated by biologists, that they will be briefly recapitulated at this place.

1. A colloidal solution consists of a fluid matrix which holds in suspension more solid or viscous granules (the colloidal par-

[1] Hardy, *Jour. of Physiol.*, 1899, XXIV., p. 172; *ibid.*, p. 288. Mann, "Physiological Histology," Oxford, 1902.

ticles), which differ from the dissolved substance in a crystalloidal solution in that they are relatively very large aggregates of molecules of the colloidal matter. These particles do not affect the osmotic pressure of the fluid matrix; while in a crystalloidal solution the solute exists in a molecular or ionic condition, and thus gives to the solution a definite osmotic pressure.

2. The physical state of the entire solution depends on the condition of these colloidal particles. When they are finely divided and separated from each other in the solvent, the colloid appears as a liquid or exists in the "sol" phase. If, however, the colloidal particles become fused, and thus lose their condition of fine suspension, the colloid becomes relatively solid, or passes into the "gel" phase.

3. The physical state of the colloidal particles and hence of the entire solution varies directly with certain external conditions. Thus the passage from the "sol" into the "gel" phase is accomplished in the following ways: (*A*) by variations in the temperature, (*B*) by chemical changes, (*C*) by the action of the electric current.

A. The physical state of the organic colloids varies directly with the temperature. If the "sol" phase is constant at the normal temperature, 20° C., coagulation gradually takes place as the temperature is lowered, until at 0° C. almost complete gelation has occurred. As the temperature is raised above the normal the fluidity of the solution is increased (by the subdivision of the colloidal particles and absorption of water), until a critical point is reached at which coagulation suddenly occurs, and the colloid goes into a condition of heat rigor.

B. A colloid may be coagulated by adding to it a solution of any electrolyte which bears an electrical charge opposite in sign to that carried by the colloidal particles. Thus Hardy found that a positively charged colloidal solution was coagulated by any electrolyte with a powerful anion or negatively charged ion, and the rapidity of the coagulation varied directly with the valence of the anion. A negatively charged colloid was coagulated by electrolytes of an opposite electrical character, or by powerful cathions. Similarly an electrolyte carrying a charge of the same sign as that of the colloidal particles causes them to subdivide

still further. As a result water is absorbed so that a liquefaction of the whole colloid occurs, as by a slight increase in the temperature. Thus cathions liquefy positively charged colloids; anions, negatively charged colloids.

C. The same conditions prevail in the reaction of colloids to the electrical current. Negatively charged colloidal particles fuse and form a "gel" around the anode, and tend to liquefy about the cathode during the passage of the current. Positively charged colloids coagulate about the cathode, and liquefy about the anode.

From the behavior of these colloidal solutions under various chemical and electrical conditions Hardy concluded, that the "sol" phase is maintained under normal conditions because all the colloidal particles carry an electrical charge of the same sign, and are thus mutually repelled, and remain in a state of fine suspension. Whenever there is introduced an electrical charge of an opposite sign, either by means of a dissociated electrolyte or by the electrical current, the charge carried by the colloidal particles is neutralized, and fusion or coagulation occurs.

The reaction to temperature variations is apparently due to the fact that, within certain limits, the kinetic energy of the colloidal particles varies directly with the temperature. A reduction of the kinetic energy causes a gradual fusion of the particles; an increase brings about a still finer state of suspension, and consequent liquefaction of the colloid through the absorption of water. The sudden coagulation at the critical point is probably due to a chemical change in the colloid itself.

The chief points of similarity between these colloidal solutions and protoplasm made apparent by Hardy's work are as follows:

1. The elementary physical structure of the two is the same. Like the colloid, protoplasm is known to be made up of two substances — (*a*) the fluid cell-sap or matrix which holds in suspension (*b*) the more solid proteid or protoplasmic particles, granules or microsomes of the morphologists. And the physical state of the protoplasm depends on the condition of these protoplasmic granules, just as the state of the artificial colloid depends

on the condition of its constituent particles. It is significant to find, that all the controversies that have been waged over the various theories of a fixed morphological structure in protoplasm have centered about the supposed unchanging physical condition of these more solid or viscous elements in the protoplasm. Now in the artificial colloids we see that their physical state varies directly with certain external conditions. The protoplasmic particles of course are vastly larger than the particles in an artificial colloidal solution, and we have no right to assume that the two are identical. But the protoplasmic granules bear the same relation to the cell-sap as the colloidal particles do to the fluid matrix, and they both respond to chemical and physical changes in a similar manner. A close comparison of the behavior of these two structural elements under various conditions will, I believe, throw a great deal of light on the physical basis of protoplasm.

Hardy observed that when a colloidal solution is exposed to the action of certain so-called fixing agents, the same structures are produced as by the action of these fixing agents upon protoplasm, *viz.*: a coagulation occurs in which the colloidal particles fuse in definite ways, producing a type of structure varying with the fixing agent employed. He therefore concluded that coagulation of the protoplasm is necessary to reveal the fixed types of structure thought by some to be characteristic of protoplasm under all conditions. Such types of structure then appeared to be in protoplasm, as in the organic colloids, merely artefacts; and Hardy argued that protoplasm in the living condition must be identical, as far as its physical structure is concerned, with the organic colloids in the "sol" phase, and that like the colloids its structure is constantly changing with the external conditions. Undoubtedly under the same external conditions, the protoplasm of different forms will show varying types of structure, as the above statement will allow. In some cases this structure may resemble a reticulum or other types described by the morphologists, but no one will now maintain that such a type is of universal occurrence. In the living protoplasm of *Paramœcium*, I have never seen a trace of a fixed structure.

These suggestions of Hardy and others upon the physical structure of protoplasm remained almost unnoticed by biolo-

gists until, very recently, attention was directed to them by the work of Loeb,[1] Mathews,[2] R. S. Lillie and others. Mathews, in particular, in his work upon the chemical stimulation of the motor nerve of the frog, has arrived at the conclusion that the protoplasm of the nerve is a colloidal solution whose particles carry a definite electrical charge, and that stimulation of the nerve is accomplished by a reversible change in the physical state of the protoplasm, analogous to coagulation. He finds that this stimulation or coagulation is effected chemically only by a series of electrolytes which agree in carrying a predominant negative charge of electricity, as would be the case in Hardy's positively charged colloidal solutions. It is only fair to say that these remarkable investigations of Mathews have been the inspiration of this work on the physical structure of protoplasm. R. S. Lillie[3] shows, in his work on the reaction of cytoplasmic and nuclear structures to the electric current, that these forms of protoplasm behave exactly as would positively and negatively charged colloidal solutions under the same conditions.

The experiments described above have shown that protoplasm reacts to various external conditions in a manner strictly parallel to the behavior of colloidal solutions in the presence of like stimuli. But meanwhile we have had but little evidence of the precise structural changes, that accompany these reactions, and are, as we have seen, the leading distinguishing feature of colloidal solutions. It was with the idea of studying these structural changes, which occur in protoplasm when subjected to various external stimuli, and of comparing them with the changes in colloidal solutions under the same conditions, that the following experiments were undertaken.

The material used has been the protoplasm of various protozoa, viz: *Paramœcium*, *Stentor*, *Amœba* and others, which are ideal objects for this study because of the abundance in which

[1] Loeb, as a result of his work on the stimulation of contractile tissue by ions, and on the toxic and antitoxic effect of ions on the duration of life of the *Fundulus* egg and frog's muscle, was the first to suggest, that the protoplasm reacts under these conditions like artificial colloids, although he has elsewhere adopted a different explanation. (See *Amer. Jour. Physiol.*, 1902, VI., p. 411.)

[2] Mathews, *Science*, 1902, XV., p. 492; 1903, XVII., p. 729.

[3] Lillie, *Amer. Jour. Physiol.*, 1903, VIII., p. 273.

they can be procured, and especially because their small size makes it possible to study the structural changes in the living protoplasm under high magnifications, which is out of the ques-question in more bulky tissues. The protozoa were subjected to thermal, chemical and electrical changes, and the structural modifications produced by these means were studied.

Structural Reactions of the Protoplasm of Protozoa to Physical and Chemical Changes.

I. *Reactions to Variations in the Temperature.*

In previous papers[1] I have described the structural changes that occur in the protoplasm of various protozoa when exposed to variations in the temperature, so that a very brief description will suffice here. We find, exactly as in the case of organic colloids, that the fluidity of the protoplasm varies directly with the temperature within certain limits. As the temperature is lowered below the normal, 20°C., a very gradual coagulation occurs, because of the decrease in kinetic energy and consequent fusion of the protoplasmic particles. This change is accompanied by a loss of water, so that at 0° C. there is produced a nearly solid opaque, spherical mass of protoplasm, which exists only in a resting condition. As the temperature is elevated above the normal, the reverse changes occur through the increase in kinetic energy acquired by the protoplasmic particles. They may be seen to subdivide further and become more widely separated through the absorption of water, so that the fluidity and motility of the protoplasm is greatly increased. These changes continue until the critical point is reached, at which coagulation suddenly occurs, and the protoplasm goes into heat rigor, probably because of some chemical change in the protoplasm itself. So closely do these structural changes agree with those that occur in artificial colloids under the same conditions, that a description of one would apply equally well for the other. Indeed, it was this striking similarity between the results in the two cases, that led me to compare the reactions of colloids and the protoplasm of

[1] Greeley, *Amer. Jour. Physiol.*, 1901, VI., p. 201; Biol. Bull., 1902, III., p. 165; *ibid.*, 1903, V., p. 42.

various Protozoa to chemical and electrical stimuli to see what light these reactions might throw on the physical structure of protoplasm.

II. *Reactions of the Protoplasm of Paramœcium and other Protozoa to Chemical Changes.*

Paramœcia reared on cultures containing bread were used chiefly in these experiments, although other protozoa were utilized for purposes of comparison. The method employed in the experiments was as follows: The solutions whose action upon the protoplasm was to be tested were made up in the proper dilutions, and distributed among Minot watch glasses holding about 10 c.c. of the solution. Pure, concentrated cultures of the protozoa were obtained, and a drop or two added to each of the dishes containing the solutions. At short intervals the protozoa were examined to observe any structural changes in the protoplasm. A 1/12 inch oil immersion was used to make out the finer details.

Great care is needed to distinguish between chemical and osmotic effects. In order to obviate the possibility of modifying the protoplasm through the extraction of water, all the solutions were used in concentrations of a lower osmotic pressure than that of the protoplasm, roughly equal to a $m/40$–$m/50$ cane sugar solution. In some cases the dilutions were very great, and, inasmuch as distilled water or any solution of a lower osmotic pressure will liquefy the protoplasm through the absorption of water osmotically, there would be danger of confusing the osmotic and chemical effects of the solutions, were it not for the fact that osmotic and chemical liquefaction can be readily distinguished by the type of protoplasmic structure produced by each means.[1]

All the chemicals used fall into two classes:

1. Those that effect the protoplasm only through the osmotic pressure of the solution.

[1] Miss Towle, in investigations now in progress at the University of Chicago, has observed that paramœcia may live indefinitely in redistilled water. It is probable that in this case, as in others mentioned in this paper, the variations in the structural reactions are due to the differences in the chemical composition of the cultures in which the paramœcia were reared. But this question of the structural effect of distilled water requires further investigation.

2. Those that modify the structure of the protoplasm independently of the osmotic pressure. The first class includes the non-electrolytes used, the second, the electrolytes.

Non-electrolytes. — The non-electrolytes used, distilled water, cane sugar and urea, affect the protoplasm of paramœcia only through the osmotic pressure of the solution. There is no chemical effect. All those solutions hypotonic to protoplasm produce a liquefaction through the absorption of water, while isotonic solutions have no effect on the structure of the protoplasm, and hypertonic solutions coagulate the protoplasm through a withdrawal of water. These effects are shown in the following table:

TABLE I.

	m/5	*m*/10	*m*/40	*m*/160
Cane sugar,	Coagulation.	Coagulation.	No effect.	Liquefaction.
Urea,	"	"	"	"

An examination of these structural changes under a high magnification makes clear the fact that these non-electrolytes modify the structure of the protoplasm solely through a change in the amount of water in the fluid matrix which holds the more viscous elements in suspension. The size of the protoplasmic particles remains unaffected. In hypotonic solutions the protoplasmic particles are more widely separated by an increase in the amount of water in the fluid matrix. In hypertonic solutions they are brought closer together, or may fuse through a withdrawal of the surrounding liquid.

Electrolytes. — As far as their effect on the physical structure of the protoplasm of *Paramœcium* is concerned, all the electrolytes used fall into two classes: first, those that coagulate even in solutions whose osmotic pressure is far less than that of the protoplasm; second, those that liquefy the protoplasm in any dilution. Moreover, all the members of the first class agree as far as their electrical conditions are concerned. They all have a predominantly powerful cathion,[1] but resemble each other in no other particular. Likewise all the members of the second class agree in possessing a predominantly powerful anion. Thus we

[1] This "predominance" may be a function of the solution tension of the ions. See Mathews, *Amer. Jour. of Physiol.*, 1904, X., p. 290.

see that anions, or negatively charged ions, liquefy the protoplasm of *Paramœcium*, cathions, or positively charged ions, coagulate without any regard for the supposed chemical affinities of the electrolyte.

That we are not dealing with a specific chemical effect for each electrolyte is still further shown by the fact that the activity of each solution is roughly proportional to the valance of the predominant ion. Thus salts containing trivalent anions or cathions are effective in much greater dilutions than bivalent or univalent salts. All these facts are indicated in the accompanying table which gives a list of the electrolytes used, their effects on the physical structure of the protoplasm, and the greatest dilution at which they are effective. The maximal dilutions can be only approximate, as the action of identical solutions is not the same on paramœcia from different cultures, because no two are exactly alike in respect to chemical composition and osmotic pressure. Especially is this true of the liquefying electrolytes, for, as we have seen, in very dilute solutions it is frequently difficult to determine the point at which the specific liquefying action of the anion ends and the osmotic absorption of water commences. As has been already mentioned, there are structural differences between these two forms of liquefaction which enable us to distinguish between them with considerable accuracy, and the fact of chemical liquefaction can be easily demonstrated by using the liquefying substances in solutions about isotonic with protoplasm. Of course no such difficulty arises with the coagulating solutions, for at great dilutions the specific coagulating action of the cathion must overcome the tendency for the protoplasm to be liquefied through the entrance of water osmotically, and there is thus a well-defined point at which the coagulation of the protoplasm by the cathion ends and its osmotic liquefaction commences.

TABLE II.

Coagulating Solutions.	Liquefying Solutions.
$m/1{,}600$ HCl, $m/1{,}600$ HNO_3	$m/1{,}600$ NaOH, $m/1{,}600$ KOH
$m/2{,}400$ H_2SO_4	$m/1{,}600$ $Ba(OH)_2$, $m/1{,}600$ $Sr(OH)_2$
$m/40$ KCl	$m/40$ NaCl, $m/40$ NH_4Cl, $m/40$ $NaNO_3$
$m/640$ $MgCl_2$, $m/640$ $CaCl_2$, $m/640$ $Ca(NO_3)_2$, $m/640$ $BaCl_2$	$m/640$ Na_2SO_4, $m/640$ $(NH_4)_2C_2O_4$
$m/320$ $MgSO_4$	$m/2{,}400$ Na_3PO_4, $m/2{,}400$ $Na_3C_6H_5O_7$
$m/1{,}600$ Al_2Cl_6, $m/1{,}600$ Fe_2Cl_6	

It will be seen by an examination of the table that the activity of any of the salts, as is shown by the strength of solution required to modify the structure of the protoplasm of *Paramœcium*, varies directly with their valence. The acids and bases are much more powerful in their action than any of the salts of a similar valence, probably because of the known disproportionate kinetic energy of the hydrogen and hydroxyl ions.

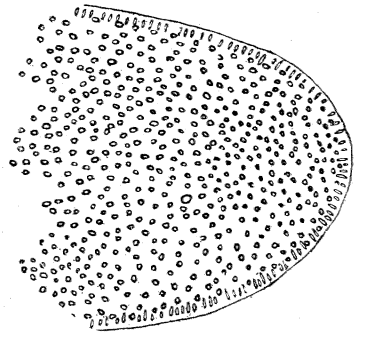

FIG. 1. Normal protoplasm of *Paramœcium*. $\frac{1}{12}$ in. oil immersion.

All the acids and salts found in the first column of the table, which agree in coagulating the protoplasm through the action of the predominant cathion, effect changes in the protoplasm so similar as to be practically indistinguishable even under a high magnification. The less active solutions, such as KCl and $MgSO_4$, do not produce quite so dense a coagulum as the others, and the reaction is considerably slower. But in all the bivalent and trivalent salts and the acids, a distinct clouding of the protoplasm can be observed within thirty minutes after the paramœcia have been immersed in the solution. This clouding of the protoplasm increases and is accompanied by a shrinking of the cell owing to a loss of water, until within a few hours, the whole cell is reduced to a subspherical mass of densely opaque protoplasm (see Fig. 2). The changes are identical with those produced by a lowering of the temperature.

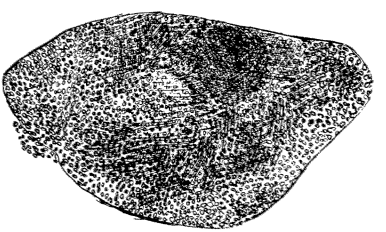

FIG. 2. A *Paramœcium* in $m/320$ $MgCl_2$, showing a typical coagulation of the protoplasm.

An examination of the protoplasm with a one-twelfth inch oil-immersion lens reveals the fact that the clouding of the protoplasm is due to a separation of the two elements of the protoplasm, the cell sap and the protoplasmic granules. These two elements lose

their state of fine mixture or suspension, which is always characterisic of motile protoplasm, and the protoplasmic particles tend to fuse into a spongy coagulum or "gel" from which the cell sap continually escapes, until a complete separation of the two elements is brought about. This fusion of the protoplasmic particles may result in the formation of two varieties of coagulated structure. The fused particles may appear as spherical bodies of proteid material, unconnected but closely massed together in such a way as to form an exceedingly dense coagulum

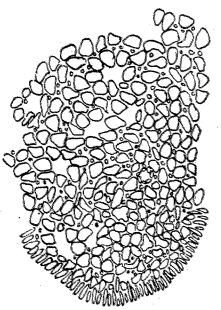

FIG. 3. Coagulated protoplasm of *Paramœcium* under $\frac{1}{12}$ oil-immersion. A dense coagulum formed after an exposure to *m*/320 $CaCl_2$ for twenty-four hours.

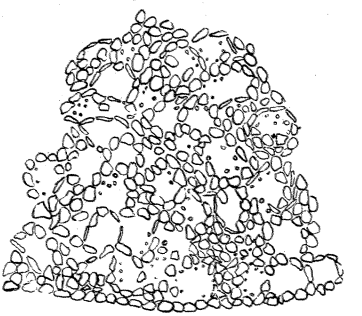

FIG. 4. Another type of coagulation viewed under the $\frac{1}{12}$ oil-immersion. The result of a ten hour residence in *m*/800 HCl, showing a trace of the reticular structure.

(see Fig. 3). Or the protoplasmic particles may fuse into fibrils or anastomosing threads which form an incomplete network, holding the cell sap in its interstices. The fibrils may eventually become thicker and transform the network into a relatively solid coagulum (Fig. 4). These last forms of coagulated structure are very similar to the network formations obtained by Hardy in organic colloids and various protoplasmic tissues by the action of fixing agents, and have been formerly supposed to be characteristic of living protoplasm. As in Hardy's experiments these fibrillar or reticular structures never appear in the living protoplasm of *Paramœcium*, but are solely an incidental result of the process of coagulation by chemical or other means.

The salts and bases in the second column of the table, which produce a common liquefaction of the protoplasm of *Paramœcium* through the action of a predominant anion, are effective like the

coagulating solutions, in dilutions which are roughly proportional to their valence.

The univalent salts have a comparatively weak effect upon the protoplasm, and relatively high concentrations are necessary before we can be sure that the liquefaction is due to chemical and not osmotic means. In solutions of the bases, bivalent and trivalent salts, however, the effects are unmistakable, and are exactly the reverse of those initiated by the coagulating solutions. Within a very few minutes after immersion in the solution liquefaction first becomes discernible as a clearing of the protoplasm. This process proceeds until the protoplasm loses its characteristically granular appearance, and becomes semi-transparent. At

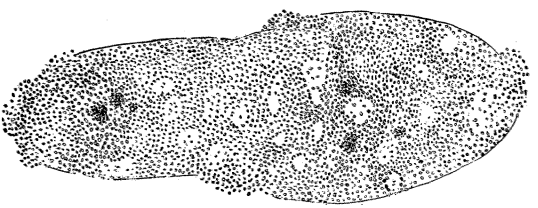

FIG. 5. A *Paramœcium* in $m/320$ Na_2SO_4, showing a typical liquefaction of the protoplasm.

the same time the cell membrane becomes greatly swollen through the absorption of water, which frequently gives the protoplasm a vacuolated appearance, and gives rise to droplets which cling to the protoplasm underneath the cell wall. The result is an irregular watery mass of protoplasm from which the solid elements have, to a superficial examination, completely disappeared (see Fig. 5). In the solutions of trivalent salts these changes occur with such violence that the cell membrane is disrupted, and the disintegrated protoplasm becomes scattered throughout the solution. Thus the liquefying anion has the same effect upon the protoplasm as a slight increase in temperature.

If, as in the case of the process of coagulation, these microscopic changes be studied under a one-twelfth inch oil-immersion, it will be at once seen that a change in the physical state of the protoplasmic particles is responsible for these profound structural modifications. The particles apparently continue to divide as the process goes on, until their size becomes so small that they

are discernible only under high magnifications. At the same time a rapid absorption of water takes place, and the particles become widely separated in the now exceedingly fluid cell sap. We thus have a means of distinguishing between liquefaction by chemical and osmotic means. As has been described above, the central feature of the process of chemical liquefaction is a splitting up of the protoplasmic particles and consequent imbibition of water through this increase in the absorbing surface (see Fig. 6). In the process of osmotic liquefaction, however, the size of protoplasmic particles remains unchanged, and water enters the protoplasm solely because of the osmotic relations between the cell-sap and the surrounding liquid. These two processes can be distinguished microscopically as indicated even under a low magnification, for paramœcia that are liquefied osmotically never lose their granular appearance, while those liquefied chemically become markedly transparent.

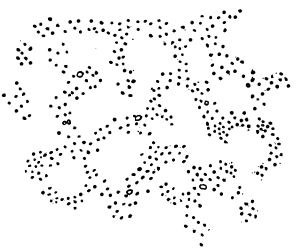

FIG. 6. Liquefied protoplasm of *Paramœcium* under $\frac{1}{12}$ oil-immersion. Formed by an exposure of two hours to $m/320$ Na_2SO_4.

That in these phenomena we are dealing also with variations in the surface tension relations of the protoplasm is apparent from the change of form which occurs during coagulation and liquefaction. The surface tension force is neutralized either by an increase in temperature or by giving all the protoplasmic particles a like charge which tends to make them mutually repellent, and thus introduces a disrupting force. Hence, if we destroy the electrical charge, we not only allow the protoplasmic particles to fuse, but we increase the surface tension. Thus, during coagulation, the cell tends to assume a spherical form which is characteristic of all resting cells. The opposite is true during liquefaction. The disrupting force is still further increased by the introduction of a charge of the same sign as that carried by the protoplasmic particles, and the cell assumes an irregular form. The most powerful anions used, the phosphate and citrate, bring about this disruption of the cell with almost explosive violence, so that the cell membrane bursts and the protoplasmic

particles are widely scattered. After a residence in these solutions of ten to fifteen minutes, no vestige of the paramœcia remains beyond the scattered protoplasmic granules.

The above experiments were repeated on *Vorticella*, *Stentor* and *Hydra*, and essentially the same structural changes were produced. The protoplasm of *Vorticella* reacts to electrolytes exactly as does the protoplasm of *Paramœcium*. In *Stentor* the differentiation between the two layers of protoplasm, endosarc and ectosarc, is more complete, and the less differentiated granular endosarc responds to the solutions in the typical manner, while the clear striated ectosarc remains practically unaffected. Thus the process of coagulation results in the formation of a cell whose endosarc is shrunken into a dense, spherical mass within the ectosarc, which retains its original form and size. During liquefaction the endosarc becomes wholly transparent, and we have produced an apparently empty cell which is bounded by the unchanged ectosarc as before. *Hydra* reacts in the same manner, the endoderm responding to the solutions as did the endosarc of *Stentor;* and we have produced animals of the original size and shape, but with either a coagulated or liquefied interior. It appears from these experiments that the physical conditions already described are applicable to protoplasm only in its primitive granular condition. The differentiation into such simple structures as the ectosarc of *Stentor*, or the ectoderm of *Hydra* has changed to some extent its elementary physical state. The high degree of viscosity of such protoplasm suggests that it is normally much nearer the "gel" phase.

Like all the typical physical modifications of protoplasm, these changes of structure are reversible, if they are stopped at the proper time. Thus coagulated protoplasm may be again liquefied by the action of a powerful anion, or liquefied protoplasm may be coagulated by a cathion. This fact seems to show with special force that the physical state of the protoplasm at any moment is the result of a definite reaction to the chemical conditions of the environment.

The whole behavior of protoplasm under the action of dilute solutions of electrolytes suggests the conclusion, that, as far as its chemical and physical reactions are concerned, it is a colloidal

solution whose particles carry a definite electrical charge. The colloidal particles in an artificial colloidal solution are apparently identical, as far as their physical reactions are concerned, with the protoplasmic or proteid particles held in the cell sap of protoplasm; at least they both react to thermal and chemical changes in the same way. The reaction to chemicals further demonstrates the fact, that, like the colloidal particles, these protoplasmic elements carry a uniform electrical charge under normal conditions; for by no other assumption can we explain the facts that salts, acids and bases modify the structure of protoplasm only by virtue of their electrical properties, and that non-electrolytes have no effect whatever beyond that of the osmotic pressure of the solution. Indeed the action is not, properly speaking, a chemical one at all, but an electrical one. In *Paramœcium* the protoplasmic particles are apparently negatively charged, and the mutual repulsion of these similarly charged particles, keeps them in a state of fine suspension. Under these conditions the protoplasm exists in a "sol" or liquid phase. Predominant cathions neutralize this negative charge; the repelling force is removed, and the particles fuse, forming a coagulum. A predominant anion further increases the disrupting force, and a state of still greater fluidity or liquefaction results.

A still closer comparison between artificial colloids and the protoplasm of *Paramœcium* is possible. It will be remembered that Hardy succeeded in reversing the charge carried by the colloidal particles by changing the chemical conditions of the solution. Thus the addition to the solution of a small amount of a base gave an alkali-modified colloid whose particles were negatively charged. Such a solution was coagulated only by cathions. Likewise the addition of a small amount of acid gave an acid-modified solution, whose particles were positively charged; this solution was coagulated by anions. How this change is effected by acids and alkalis we cannot say, but certain it is that the electrical nature of the colloid is determined by certain ions present in its fluid matrix.

The reactions of the protoplasm of paramœcia to electrolytes described above, occurred in organisms which had been reared in a normal, alkaline culture. It was found that when the culture

medium became acid through the fermentation of bread, the structural reactions of the protoplasm to electrolytes was changed. And they were changed in such a way as to suggest that there had been a reversal of the electrical charge carried by the protoplasmic particles, or in other words, that there had been formed an acid-modified protoplasm. Thus the protoplasm of paramœcia from an acid culture was not coagulated by cathions and liquefied by anions, as was the case without exception with paramœcia from an alkaline culture, but the reactions were as follows: The first change produced by the neutralization of the normal alkalinity of the culture was an irregularity in the structural reactions. Instead of all the organisms being coagulated by cathions and liquefied by anions, some were coagulated, and some were liquefied in each solution, apparently indicating a condition in which the protoplasm of some of the paramœcia had been modified by the acid, the rest being still unaffected. As the acidity of the solution increased, the number that were coagulated by anions and liquefied by cathions correspondingly increased until, in a few instances, a complete reversal of the structural reaction to electrolytes was produced. While the reversal was more often not complete, in all cases it occurred in a varying proportion of the paramœcia from acid cultures, while the remainder were generally rendered indifferent to the solutions which formerly coagulated or liquefied them. The complete reversal occurred only in the salt solutions. The acids always coagulated the protoplasm regardless of the character of the culture, and to alkalis, the paramœcia from acid cultures were only rendered indifferent. All these results indicate that the particles of the acid-modified protoplasm have become positively charged like the particles in Hardy's acid-modified colloids.

The view that this change in the structural reactions of paramœcia is due to a modification of the protoplasm by acids is rendered more probable if we follow the reactions of paramœcia from day to day, which are taken from a culture which is gradually becoming acid by the fermentation of bread. In such a case we can easily see the gradual fading out of the normal reaction to electrolytes, and the assumption of the one peculiar to acid-modified protoplasm. This change in structural reaction always

accompanies the neutralization of the alkalinity of the culture. The complete reversal in the sign of the charge carried by the protoplasmic particles is apparently too fundamental a change to occur without killing the protoplasm except under the most perfect conditions. While much more work must be done on this point, we can assuredly say that the normal electrical charge carried by the protoplasm of *Paramœcium* bears a very close relation to certain chemical conditions of the environment, of which the alkalinity of the surrounding medium may be taken as one of the most important. These results are especially significant in the light of the behavior of paramœcia from various cultures toward different forms of stimuli, as we shall see when we discuss this subject.

III. *Reactions of the Protoplasm of Paramœcium to the Electrical Current.*

The above conclusions relating to the ultimate physical structure of protoplasm and the electrical conditions underlying it, are further borne out by a study of the effects produced on the structure of the protoplasm by the constant current. It has long been known that the constant current has a profound polar effect on the protoplasm of various protozoa.

Thus Kühne, Verworn[1] and others have shown that when a protozoan is exposed to the action of a weak current, a contraction occurs on the anodal side of the cell, and a relaxation on the cathodal side. This phenomenon was first described for *Actinosphærium*, a large heliozoan with many radiating pseudopodia. Very soon after exposure to the current the pseudopodia on the anodal side become contracted into irregular shapes, and are finally completely withdrawn into the cell, while those on the cathodal side remain fully extended. *Amœba* is still more sensitive to the current. The whole cell contracts on the anodal side while pseudopodia are rapidly thrown out toward the cathode so that the animal moves in this direction. The same general changes have been observed in *Paramœcium*[2] and many other protozoa. If the organisms are exposed to the current for a

[1] Verworn, *Arch. f. d. ges. Physiol.*, 1889, XLV., p. 1.
[2] Pearl, *Amer. Jour. of Physiol.*, 1900, IV., p. 96.

longer time, more pronounced structural changes ensue. The contraction on the anodal side continues until the protoplasm at this point can be seen to disintegrate, forming dense granular masses, while on the opposite side the protoplasm becomes even more clear and transparent than it was at first, frequently flowing out over this portion of the cell in irregular liquid masses. The observations have been repeated in a large number of forms so that the facts of definite polar modifications in the structure of the protoplasm during the passage of a weak constant current seem to be very well established. At the same time it has been shown in many forms that there is a movement of the protoplasmic particles away from the cathodal side where liquefaction of the protoplasm is taking place toward the anodal region of the cell where the disintegrating or "etching" effects are shown.[1]

I have repeated these experiments and have furthermore shown that when paramœcia are isolated in a weak gelatine solution or held in a fine mesh-work of cotton during the passage of the current so that they are unable to move freely from pole to pole, a slightly different structural reaction to the current is obtained after the current has been passed for from three to five hours. In this case all those paramœcia which are held in the region of the anode are modified as was the anodal side of the cell in the preceding experiment, *i. e.*, the whole cell contracts into a dense, opaque mass of protoplasm. Likewise the paramœcia held about the cathode are modified like the cathodal side of the cell in the former experiment, *i. e.*, the cell contents are liquefied and there is formed a large transparent cell of fluid protoplasm which ultimately bursts because of the increased pressure on the cell wall. Thus we have the same structural changes occurring either in the whole cell immediately about the anode, or in the anodal side of any of the cells exposed to the current; opposite changes occurring in the cells about the cathode or on the cathodal side of all the cells in the preparation.

A microscopical examination shows that these changes are

[1] Wallengren (*Zeitschr. f. allg. Physiol.*, 1903, III., p. 22) states that in the Rhizopoda only can the movement of the protoplasmic particles toward the anode be demonstrated. In the Infusoria the constant current does not affect the normal streaming of the protoplasm.

identical with the structural changes produced by the action of electrolytes upon the protoplasm. About the anode and on the anodal side of the cells the protoplasm is coagulated as by the use of cathions in weak solutions; about the cathode and on the cathodal side of the cells, the protoplasm is liquefied as by the use of anions in weak solutions. Not only are these structural effects the same, but they can be shown to be produced by the same means in each case, *i. e.*, by electrically charged ions, which are present in very dilute solutions of electrolytes, as we have shown, and which serve to carry the current from pole to pole when it is passed through such weak salt solution as the culture media of paramœcia which were used in the experiment.

It was formerly supposed that these polar effects were due to the formation of acids on the anodal side of the cell and alkalis on the cathodal side, but we have no satisfactory evidence for such internal polar changes. During the passage of the current, the anions prevail about the cathode, and are constantly diffusing toward the anode. During this passage they are continually impinging on the cathodal side of the paramœcia, hence a liquefaction of the protoplasm takes place at these places. Cathions prevail about the anode, and continually impinge on the anodal side of the cells in their diffusion toward the cathode so that coagulation occurs at these points. In either case the structural changes are produced by virtue of the electrical charge which the ions carry, and not by any specific chemical effect of the ions or molecules. We thus see that the chemical and electrical means of modifying the protoplasmic structure are identical.

It will be remembered that these changes in the structure of the protoplasm produced by solutions of electrolytes or the electrical current are the same as those which are brought about in organic colloids by the same means. The alkali-modified colloids in Hardy's experiments were coagulated by cathions in solution or at the anode during the passage of the current. They were liquefied by anions or at the cathodes. It is interesting to note also that in the protozoa a movement of the protoplasmic particles within the cell toward the anode has been observed, which corresponds exactly with the movement in the same direction among the colloidal particles of an alkali-modified colloid. Like

the colloidal particles, they always move toward the point at which coagulation occurs.

All these experiments seem to show that the structural changes produced in protoplasm by thermal, osmotic, chemical or electrical changes are the same, because all of these variations in the external conditions act upon protoplasm only by altering the physical state of its solid elements. Thus in the case of *Paramœcium*, at least, the structure of the protoplasm is seen to be not fixed and uniform, but to depend directly on certain external conditions and to vary with their variations. The best expression of this behavior of protoplasm is found in the laws of the reaction of colloidal solutions to external conditions.

Effect of these Structural Modifications on the Vital Properties of the Protoplasm.

Having determined the changes produced in the structure of the protoplasm by various chemical agencies, it remained to ascertain how these structural changes modify the vital properties of protoplasm. How far may a particular state of protoplasmic activity be correlated with a given physical condition of protoplasmic structure? Or does the reaction of an organism as a whole to an external stimulus depend in any measure upon the effect that stimulus may have on the structure of the protoplasm?

I. Growth and Cell Division.

The rate of cell division may be taken as the best indication of the general protoplasmic activity among the Protozoa, after the method adopted by Calkins[1] in his work on the "Life Cycle of Paramœcium." A quickened rate of cell division means an increase in the metabolic activities of the cell. A condition of slow metabolism is indicated by the cessation of cell division and the transformation of the motile cell into a spore or cyst or other resting stage. It has been already shown, in a paper[2] on the reactions of various protozoa to variations in the temperature, that precisely those temperature conditions, which liquefy the protoplasm, stimulate cell division, and those temperatures which coagulate

[1] Calkins, *Arch. f. Entwickelungsmech.*, 1902, XV., p. 139.
[2] Greeley, *Amer. Jour. Physiol.*, 1902, VI., p. 122.

the protoplasm inhibit it. Thus the rate of cell division increases steadily with a slight elevation of temperature above the normal until the critical coagulating point is reached. A lowering of the temperature progressively decreases the rate of cell division, until the point is reached at which it ceases altogether, and the protoplasm goes into a resting condition.

The same relation between the rate of cell division and the physical state of the protoplasm is found to hold good also as a result of the reactions of the protoplasm of *Paramœcium* to solutions of electrolytes.

With paramœcia from alkaline cultures, anions or liquefying agents stimulate cell division, cathions and coagulating agents inhibit it. Thus I have frequently observed in my experiments that when the liquefying solution is too weak seriously to modify the structure of the protoplasm, it will, however, greatly increase the motility of the protoplasm and the rate of cell division. Since the size of the paramœcia remains uniform, it follows that this must indicate also increased growth and general metabolic activity. In the coagulating solutions, on the contrary, there are produced spherical resting cells that greatly resemble spores. This antagonism between these two classes of solutions is still more clearly shown by the fact that the anions greatly accelerate the germination of spores, or the passage from a resting into a motile condition. In these solutions the spherical form of the spore is soon lost through a neutralization of the surface tension. This has been shown in the case of some of the monads and fresh-water algæ.

R. S. Lillie[1] has shown that a decrease in the surface tension must accompany cell division, and that this is accomplished in the case of certain marine animals by the electrolytes present in the sea water, for if these electrolytes be withdrawn, cell division not only stops, but a partial fusion of the already formed blastomeres occurs. It appears that the surface tension relations are very important in all these protoplasmic reactions to external conditions. Protoplasmic movement, cell division and growth all occur in opposition to the surface tension force.[2] Consequently any external condition which neutralizes the surface ten-

[1] Lillie, BIOL. BULL., 1903, IV., p. 164.

[2] See Spaulding, BIOL. BULL., 1904, VI., p. 97.

sion accelerates these expressions of the general activity of the protoplasm. These conditions are those which bring about a liquefaction of the protoplasm, so that the protoplasmic activity is seen to vary directly with the amount of water the protoplasm contains. Conversely the assumption of a quiescent spherical resting stage in various protozoa is the result of an increase in surface tension, and is formed by those conditions which cause a coagulation of the protoplasm and a loss of water. Thus a slight increase in the temperature is seen to have the same effect on these simple protoplasmic properties of *Paramœcium* as anions ; a lowering of the temperature acts like cathions ; since each set of conditions produces the same structural effects.

II. *The Tropisms.*

We have at the present time an enormous amount of information concerning the reactions of organisms to external stimuli, but we know almost nothing of the physical or chemical effects of these attractive or repellent agents on the protoplasm of the organism, and consequently we are not able to offer any satisfactory explanation of the mechanism of the tropic response. In the following experiments I have studied the reactions of paramœcia to thermal, electrical and chemical stimuli, and have attempted to show that the reaction of *Paramœcium* to each stimulus depends on certain structural changes in the protoplasm, which are a result of the stimulating action.

1. *Thermotaxis.* — The reactions of paramœcia to variations in temperature are exceedingly definite. It has been stated by many observers that they are positive to temperatures between approximately 23° and 27° C., and are negative to all others. This reaction is beautifully demonstrated by placing the paramœcia in a long, narrow dish which is heated at one end and cooled at the other. It has been already shown that those temperatures to which *Paramœcium* is positive constitute exactly those thermal conditions which bring about a liquefaction of the protoplasm and a reduction in the surface tension. Those temperatures to which *Paramœcium* are negative, coagulate the protoplasm. We thus see that, in the case of thermotaxis, attraction is accompanied by a liquefaction of the protoplasm, repulsion by coagulation. The

extreme delicacy of the adjustment between the physical structure of the protoplasm and the external conditions has hardly been recognized. For example, the smallest perceptible elevation of temperature above the normal results in a decided increase in the fluidity of the protoplasm, and it is probable that this structural change explains the extreme sensitiveness of the paramœcia, judged by their thermotropic reactions, to these same variations in the temperature.

2. *Galvanotaxis.* — It has been well known that paramœcia normally react to the electrical current in a vigorous and definite manner by orienting themselves with their anterior end toward the cathode, and swimming rapidly in this direction, so that eventually a dense gathering of the organisms occurs about the negative electrode. In other words they collect at that point in the electrical field where the conditions are such as to induce a liquefaction of the protoplasm. After a weak current has been passed through the preparation for from thirty minutes to one hour, it will be seen that the dense gathering at the cathode begins to break up and a reverse movement toward the anode sets in. The number of paramœcia that exhibit this reverse movement varies with the conditions of the organisms at the time of the experiments, as will appear later; but with paramœcia from alkaline cultures only a small proportion of the entire number will be seen to swim toward the anode at any one time. At first the paramœcia swim only a short distance toward the anode and then immediately dart back to the cathode, but the length of the reverse reaction increases until a few reach the anodal end of the dish. Having arrived at the anode, they immediately swim back to the cathode again, and no gathering occurs at the anode except in rare cases after the current has been passed for about two hours. The paramœcia normally keep the anterior end pointed always toward the cathode, so that they swim backwards toward the anode. But this is not always the case.

After a large number of experiments with paramœcia under various conditions, I find that the relations between this initial and secondary reaction to the current may be greatly modified. With paramœcia from alkaline cultures, the secondary reaction begins only after the current has been passed for thirty minutes

or more and is at first of an exceedingly transitory nature. But with paramœcia that have been reared in a culture that has been made slightly acid by the fermentation of starch, and hence are in an acid-modified condition as before described, the secondary reaction may begin as soon as the paramœcia reach the cathode. In these cases no gathering occurs at the cathode but each *Paramœcium* immediately reverses the stroke of the cilia and swims back to the anode. The process is repeated at that point, so that we have for a time no collection at either pole but a continuous line of paramœcia swimming in either direction until eventually they come to rest in about equal numbers at each end of the preparation. Under the most favorable acid conditions a number of paramœcia, varying from one to fifty per cent. of the whole number, exhibit an initial reaction toward the anode and a secondary reaction toward the cathode, while the remainder react in the manner described above.

That this immediate reversal of the normal reaction and the initial response toward the anode are due to the acidity of the culture medium may be shown by the following experiments. If 5 c.c., of a neutral [1] culture of paramœcia be isolated and tested to the current, it will be found that they all exhibit the characteristic response toward the cathode and form a dense gathering at that point. If now, however, from two to four drops of an $m/10$ solution of hydrochloric or other acid be added to the culture, and, after standing for thirty minutes, the reaction to the current be again tested, it will be found that either an initial or an immediate secondary reaction toward the anode has set in. Also the addition of a small amount of acid to an already acid culture invariably strengthens the anodal response of paramœcia. Likewise in every case in which I have tried it, the neutralization of the acid with NaOH, or the addition of the solution of a salt with a trivalent anion like Na_3PO_4 or $Na_3C_6H_5O_7$ entirely destroys both the initial and the secondary response toward the anode, and leaves only the characteristic gathering at the cathode.[2]

[1] It is necessary to use paramœcia from an approximately neutral culture for this experiment. The normal reaction toward the cathode is too firmly fixed in paramœcia from a strongly alkaline culture to be reversed.

[2] More striking results have been obtained with *Volvox*. After an exposure of half an hour or more to a slightly acid medium, practically every organism completely reverses its response, so that a dense gathering is formed about the anode.

In several instances, after a prolonged exposure to a medium which had been acidulated by the addition of hydrochloric or acetic acid, the paramœcia exhibit still another form of reaction. In these cases the paramœcia orient themselves transversely or slightly obliquely to the direction of the current, and swim very slowly from side to side of the preparation. At the same time, however, the organisms appear to drift passively toward the anode. I observed this form of reaction only about half a dozen times, but on each occasion the reaction was exceedingly well marked. A reversal of the current caused an instant reversal, of both the direction of the swimming and the passive drifting of the organisms. Notwithstanding the peculiar orientation of the paramœcia, they all tended to form a gathering about the anode under these acid conditions.

It has been observed by Loeb, Jennings and others that the addition of other substances, like NaCl, to the solution containing the paramœcia will cause a reverse movement toward the anode. I investigated this question and found that the addition of almost any salt in sufficient quantities to extract water from the protoplasm will cause a more or less complete reversal. A large number of salts, of both positive and negative electrical conditions, were used, and the effect was seen to be purely osmotic in character, except in the case of the salts with trivalent anions or cathions. The former, as the phosphates and citrates, act like the hydrates in very weak solutions and completely destroy all traces of a response toward the anode. The latter, as Al_2Cl_6, produces an almost instant coagulation of the protoplasm and hence bring about the same effect as the extraction of water osmotically. It has been already stated that a lowering of the temperature coagulates the protoplasm, and it is interesting to note, that a partial reversion of the electrical response may be produced by this means also. After the paramœcia have been exposed to a temperature of 2° to 3° C., for one hour or more, the normal response is entirely lost, and a slight movement toward the anode can be detected.

Our ignorance of the precise nature of the ciliary response is too great to allow even an attempt at an explanation of the mechanism of this response to the electrical current. We will

first have to obtain a satisfactory explanation of the rhythmical contraction of the ectosarc which controls the cilia. It is certain that the surface tension relations of the muscular elements play an important rôle in this process. In *Amœba* the problem is very simple. The protoplasm contracts on the anodal side because of the neutralization of the charge carried by the protoplasmic particles and consequent increase in surface tension. Pseudopodia are thrown out on the cathodal side, and movement occurs in this direction because of the decrease in surface tension at this point. But for paramœcia it suffices at present to show that the sense of the response is not a fixed attribute of the organism which has been acquired by natural selection, but that it is a purely physical response to an external stimulus, and varies directly with the conditions under which it occurs. The ultimate determining factor of the response to the electrical current must be the electrical conditions of the protoplasm itself. In paramœcia from a normal alkaline culture the protoplasmic particles appear to be negatively charged. These paramœcia collect about the cathode where liquefaction of the protoplasm occurs. But with precisely the same conditions, under which liquefaction is produced not by anions but by cathions, *i. e.*, an acid culture medium, we find that the characteristic gathering about the cathode does not occur, but the paramœcia tend to move toward the anode where the protoplasm would now become liquefied. The only explanation of this phenomenon that presents itself is the assumption, that in an acid medium the protoplasmic particles become positively charged in a portion of the organisms (for the reaction is never completely reversed). The partial reversal of the reaction by osmotic means is also due to an alteration in the electrical conditions of the protoplasm, as has been shown.

Hardy's acid and alkali-modified colloids reacted also in an opposite manner to the electrical current because in the acid solution the particles were positively, and in the alkaline solution negatively charged; and while the explanation is not so simple in the case of *Paramœcium*, because movement is effected by a complex motor apparatus, still the sense of the reaction must be ultimately due to the same cause in both cases, *i. e.*, the charge carried by the colloidal or protoplasmic particles. Moreover,

Hardy observed the same secondary reversed movement of the colloidal particles as has been observed in the reaction of *Paramœcium*. All these facts make very evident the similarity which exists between the electrical conditions in the two solutions. Lillie[1] has observed a similar relation between the response to the electric current and the chemical condition of the protoplasm whether acid or alkaline, in his experiments upon nuclear and cytoplasmic structures cited at the beginning of the paper. He shows, that, when exposed to the electric current, nuclear structures, which contain a large amount of nucleic acid, move toward the anode, while cells very rich in cytoplasm, which is basic in reaction, move toward the cathode.

3. *Chemotaxis.* — A large number of important contributions have been made during the last few years to our knowledge of the reaction of protozoa to chemical stimuli. Most notable have been the remarkable series of investigations carried on by Jennings, who has given us not only a complete account of the sense of the reaction of many protozoa to a wide range of chemical substances, but also an accurate description of the method of the reaction in each case. My own results agree with those of Jennings in all essential points. I confirm his account of the "motor reaction" of *Paramœcium* when under a chemical stimulus. It was not my purpose to repeat any of Jennings' experiments, but only to ascertain the chemotropic reactions of *Paramœcium* under various conditions, to see if they could be modified by external influences as was the galvanotropic response. The only respect in which my conclusions depart from Jennings' is that my experiments seem to show that the chemotropic reactions of *Paramœcium* which he describes are not of universal occurrence, but limited to paramœcia which have been reared under definite chemical surroundings. In other words, the sense of the chemotropic reaction, like the galvanotropic, depends upon certain chemical conditions of the environment.

Jennings found that paramœcia were in general positive to weak acids (*i. e.*, formed a gathering within the drop) and negative to weak alkalis. He also observed that they reacted in a constant manner to a large number of salts. In my own experi-

[1] Lillie, *loc. cit.*

ments, I find, that only those paramœcia which have been reared in a slightly acid culture medium are positive to acids, and that paramœcia from clear alkaline cultures are negative to acids and positive to alkalis. It appears also that salts with trivalent cathions act like acids, and salts with trivalent anions act like alkalis. No definite conclusions could be drawn from the action of the univalent and bivalent salt solutions. For the purpose of the experiments, we are most vitally concerned with the reaction of those solutions which carry the heaviest positive or negative charges of electricity.

The greatest caution is needed in determining the acidity or alkalinity of the culture medium. A carefully prepared litmus solution is the best indicator. Phenol-thalein may also be used for the detection of small amounts of alkalis. Paramœcia freshly reared in a culture whose alkalinity has been determined in this way invariably react to the solutions used as follows: To $m/200$ HCl, HNO_3, H_2SO_4, and acetic acid; $m/800$ Al_2Cl_6 and Fe_2Cl_6, they are negative. To $m/200$ NaOH, KOH, $Ba(OH)_2$ and $Sr(OH)_2$; $m/480$ $Na_3C_6H_5O_7$ and Na_3PO_4, they are positive. The positive reaction is shown by the organism swimming into the drop, and then giving the motor response, described by Jennings, when they come in contact with the outside water, so that a gathering is formed within the drop. The solutions to which the paramœcia are negative provoke the motor response when the organisms first come in contact with the outer edge of the drop, with the result that the solution is left empty. In some cases the paramœcia appear to be entirely indifferent to a solution, and swim in and out in an undisturbed manner. Such a reaction will also be classed as negative.

If the paramœcia from the same alkaline culture be tested from day to day as the alkalinity is being gradually neutralized by the formation of acid during the fermentation of bread, or by the addition of free acid to the culture, it will be seen that the response to these solutions slowly changes, until finally the chemotropic reaction is completely reversed, and now in the acid medium the paramœcia are positive to acids and salts with a trivalent cathion, and negative to alkalis and salts with a trivalent anion. Likewise, if the culture be again made gradually alka-

line, the first form of reaction, characteristic of alkaline cultures, returns, so that it becomes evident that the sense of the chemotropic reaction depends directly on certain chemical conditions of the surrounding medium. The transformation from the first form of reaction to the second, and *vice versa*, is a very gradual one, so that it is not immediately effected by the chemical change in the surrounding medium, and a considerable time may elapse between the neutralization of the alkalinity of the culture, for example, and the loss of the positive response to alkalis ; but eventually the reaction occurs as has been described. Thus paramœcia are seen to seek out those chemical conditions which bring about a liquefaction of the protoplasm. The sense of this response also is apparently determined by the electrical condition of the protoplasm.

An interpretation of the mechanism of this response to electrolytes is as impossible as it was in the case of the reaction to the electric current. But, since in each case the paramœcia collect under the same electrical conditions, both responses must be ultimately due to the same reaction of the contractile layer of the protoplasm to electrically charged ions, and this reaction must consist largely in the effect which the electrically charged ions have upon the surface tension of the contractile elements of the protoplasm. Experiments upon *Amœba* bear out this hypothesis. Cathions always produce a contraction of the protoplasm, while anions produce a relaxation or the extension of pseudopodia, because the former increase the surface tension of the protoplasm while the latter neutralize it. Thus *Amœba*, like *Paramœcium*, is positive to predominately negative solutions, but in the one case the response is accomplished by the immediate effect which the anions have upon the surface tension of the protoplasm, while in the other case it is brought about through the agency of a complex motor apparatus.

Conclusions.

We see that precisely those chemical changes in the surrounding medium, which modify the structural reactions of the protoplasm of *Paramœcium* to solutions of electrolytes, modify also the reactions of the organism to electrical and chemical stimuli.

The protoplasm of paramœcia from an alkaline culture is liquefied by temperatures between 23° C. and 30° C., by anions, and at the cathode during the passage of the constant current. The paramœcia also react positively to all these chemical and physical conditions. The protoplasm of the same paramœcia is coagulated by temperatures below 20° C. and above 30° C., by cathions, and at the anode during the passage of the current. The organisms are negative to all these conditions. The structural changes produced by electrolytes are partially reversed in paramœcia from a slightly acid culture, and the reactions of the organisms are also partially reversed to the electric current, completely so to solutions of electrolytes. In every case the reaction of a *Paramœcium* to an external stimulus leads it to remain under those conditions which liquefy the protoplasm. Attraction is accompanied by liquefaction, repulsion by coagulation. As far as the physical structure of the protoplasm is concerned, the conclusion from these facts seems to be that the protoplasmic particles are physically identical with colloidal particles. Hence the protoplasm of *Paramœcium* is essentially a colloidal solution whose particles carry a definite charge of electricity. The sign of this charge appears to depend on certain external chemical conditions, of which the alkalinity of the surrounding medium may be taken as one of the most important. The sign of this charge is seen to determine not only the structural modifications of the protoplasm, but also the reactions of the paramœcia to chemical and electrical stimuli. This conception of the physical structure of protoplasm is also used to explain the effect of external conditions on the processes of cell division, growth and movement through the operation of the laws of surface tension.

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